thereby delaying a definitive diagnosis of aspergillosis and initiation of specific anti-fungal therapy in the patients.

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Microfouling of manganese-oxidizing bacteria in Tuticorin harbour waters

S. Palanichamy, S. Maruthamuthu*, S. T. Manickam and A. Rajendran#

Offshore Platform and Marine Electrochemistry Centre, Central Electrochemical Research Institute Unit, Harbour Area, Tuticorin 628 004, India
*Central Electrochemical Research Institute, Karaikudi 630 006, India

Implication of manganese-oxidizers in corrosion of various alloys stimulated the investigators to concentrate on these aspects. In the present study, an attempt was made to bring out the bacterial genera involved in the oxidation of manganese in biofilms. The materials immersed in sea water for biofilm formation included polyvinyl chloride (PVC), stainless steel (SS), brass and copper. The biofilm samples were analysed quantitatively and qualitatively for both heterotrophic bacterial population (HB) and manganese-oxidizing heterotrophic bacterial population (MHB). Both qualitative and quantitative examination of biofilms showed relatively poor population density on copper. Qualitative examination revealed the representation of both Gram-positive and Gram-negative bacteria on all materials. However, only Gram-positive groups, especially of the endospore-forming genus *Bacillus* and non-endospore forming genus *Propionibacterium* were observed on copper coupons. Gram-positive genera dominated over Gram-negative genera in most of the biofilms studied. The genera identified under manganese-oxidizing bacterial isolates were *Bacillus*, *Staphylococcus*, *Synecoccus*, *Propionibacterium*, *Micrococcus*, *Pseudomonas* and *Vibrio*. Among them, *Bacillus* species was most commonly encountered in all the materials studied. Potential measurements for SS316 showed positive shift. Analysis revealed enormous amount of manganese in the biofilms.

THE affinity of marine bacteria for surfaces was first studied by Zobell[1] who demonstrated that some bacterial cells approach a surface, adhere rapidly to it, initiate glycocalyx (exopolysaccharide) production and form the discrete microcolonies that are the basic organizational units of biofilms. Costerton and Lewandowski[2], thus defined the biofilms as a matrix – enclosed bacterial populations adhere to each other and/or to surfaces or interfaces. The type and the rate of bacterial adhesion influence the nature of the surface concerned, which subsequently leads to corrosion of the material. Sulphate-reducing bacteria and iron-oxidizing bacteria have long been considered as major contributors to corrosion. Recently, manganese-oxidizers have also been identified as major contributors to corrosion[3–5]. Following the first report of Mollica and Trevis[6] to show ennoblement of

#For correspondence. (e-mail: ttn-sohum@sancharnet.in)

stainless steel (SS) exposed to sea water, there has been a growing body of literature pertaining to the ennoblement of metals[7–12]. Johnsen and Bardal[13] and Dexter and Lin[14] have related this process to the increased current density requirement for cathodically protecting stainless alloys. This process increases the pitting probabilities of SS. Maruthamuthu *et al.*[12] observed appreciable positive shift in corrosion potential in the dark rather than in the ennobling potential values recorded under daylight. Dickinson *et al.*[4] were perhaps the first to identify the deposition of MnO_2 on SS. They attributed it to ennoblement (increase in potential). It has been found that the actively metabolizing manganese-oxidizing bacteria deposit MnO_2, creating a anaerobic zone within it. This would favour the growth of sulphate reducers whose activity at low values of electrochemical potential might further stimulate the corrosion process initiated by the MnO_2 ennoblement. Although many investigators[15–17] proposed several mechanisms on the role of manganese-oxidizers on SS, they did not identify these bacteria in biofilms. In the present study, manganese-oxidizing bacteria, essentially hetertrophic in nature, isolated from biofilms that were formed on various materials exposed at Tuticorin harbour waters are identified and reported from Indian waters.

PVC coupons (15 cm × 10 cm) were degreased and rinsed with distilled water. Stainless steel (SS316) coupons (50 mm × 25 mm × 2 mm) were polished with 400 grit emery and prepassivated with 10% nitric acid at 60°C for 30 min. Brass and copper coupons (15 cm × 10 cm) were pickled in 10% HCl and polished by fine grades of emery paper (400 grit), washed with detergent, rinsed with distilled water, dehydrated in methanol and stored in a desiccator until use.

The coupons of PVC, brass and copper were immersed one metre below water surface mounting on wooden rafts. The SS316 coupons were half-immersed in sea water in fibre reinforced polymer (FRP) tank. Insulating lacquer prevented bimetallic contact at the electrical lead or coupon junction. Further, use of washer to keep the metals fixed to the frame was avoided. The sheathed wire leads which passed through holes on the wooden rafts were kept in position using an adhesive. It was possible to keep the coupons in suspension, thus preventing any contact with non-metallic support materials.

The metal coupons, copper and brass, were exposed for a period of six months (August 2000–January 2001). The PVC and SS coupons were suspended in natural sea water for a period of one month (January 2001). Except SS316, all other coupons were immersed under natural conditions below the OPMEC platform. Potentials were recorded for SS316 coupons immersed in natural sea water and in sterile sea water, with reference to a saturated calomel electrode (SCE) positioned close to the coupon.

Bacterial slime samples generated on the exposed coupons were sampled in sterile condition and immediately transferred to sterile 1% peptone saline water. All the samples were serially diluted (10-fold) using sterile 20 ppt of sea water. For quantitative examination of the bacterial colonies, the samples were inoculated by spread-plate method. The Zobell marine 2216E agar medium was used to enumerate the total heterotrophic bacteria (HB) and K-medium[18] was used to enumerate the manganese-oxidizing heterotrophic bacteria (MHB). Average bacterial counts of the replicates were determined. Morphologically dissimilar colonies were randomly selected and isolated from Zobell marine 2216E agar medium and K-medium and were streaked onto the fresh medium, respectively, to obtain pure cultures. These cultures were maintained in slants at 4°C for bacterial characterization.

The characterization of bacteria up to generic and species level was done according to the key described in *Bergey's Manual*[19] and other developed schemes[20]. For characterization of bacterial strain, a loop full of bacterial culture was inoculated into sterile nutrient broth for HB and K-medium for MHB and incubated overnight. The fresh overnight culture was subjected to microscopic, physiological and biochemical tests for presumptive identification.

Manganese level in sea water was estimated using atomic absorption spectrometer (GBC 906AA) following APDC-MIBK pre-concentration technique[21], while biofilm samples were oven-dried at 110°C and subjected to acid digestion[22], and extracts were analysed for Mn in AAS.

In general, potentials of exposed material to natural sea water could be influenced (normally upward shifting of potential/increase of potential) by several biotic and abiotic factors, which is considered to be a sign of corrosion. In the present study an attempt was made to bring out the influence of biotic factor (microbes) on the potential values of SS316 as to establish microbial corrosion by manganese-oxidizing bacteria. Potential vs time plots for SS316 alloy in the presence and absence of biofilms are shown in Figure 1. In the absence of biofilm the corrosion potential was unaffected, whereas in the presence of biofilm, the potential rapidly increased (ennoblement) up to the seventh day and thereafter a gradual increase of potential was seen. A high value of 242 mV vs SCE was recorded on the twelfth day.

Table 1 shows the average population of HB and MHB in various immersed materials. The population of HB and MHB on PVC was found to be 3.07×10^8 CFU/cm^2 and 2.36×10^8 CFU/cm^2, respectively, while on SS the population of HB and MHB was found to be 3.4×10^5 CFU/cm^2 and 1.07×10^5 CFU/cm^2, respectively. The lower population recorded on SS may be due to the nature of the surface of the material. On copper, the count of HB was 2.04×10^2 CFU/cm^2 and MHB was 1.02×10^2 CFU/cm^2, while the counts of HB and MHB on brass. were 3.9×10^6 CFU/cm^2 and 1.84×10^6 CFU/cm^2 respectively. The CFU value was relatively higher on PVC though it

was exposed for a short period of one month. This may be due to the non-toxic nature of the substratum. Brass also exhibited higher population when compared to copper. The least population density observed on copper coupons could be due to its toxic nature. Ponmariappan *et al.*[23] also reported less heterotrophic bacterial population on copper than Monel in Tuticorin harbour waters. The load of MHB was more or less the same as HB in all materials. It indicates that most of the HB act as manganese depositors after oxidation.

Table 2 shows the generic composition of HB isolated from biofilm samples scrapped from various materials. Occurrence of both Gram-positive and Gram-negative groups was noted on all the materials studied, except on copper where the Gram-negative group could not be recovered. It could be noted that Gram-positive group was found to be dominant on all the materials studied. However, on brass coupon the Gram-negative group was found to be predominant over the Gram-positive group. The genera identified under Gram-positive were *Staphylococcus* and *Micrococcus*, while the Gram-negative strains were identified as *Pseudomonas*, *Acinetobacter* and *Proteus*. The generic composition was found to vary from material to material. In PVC, the genera identified under Gram-positive included *Staphylococcus* and *Micrococcus*, while the Gram-negative groups were identified as *Pseudomonas* and *Acinetobacter*. In stainless steel coupons, the identified genera of Gram-positive bacteria were *Micrococcus* and *Staphylococcus*, while the Gram-negative strain was represented only by *Pseudomonas*. On the contrary, in brass the Gram-negative strains were found to dominate over the Gram-positive by the genera *Pseudomonas* and *Proteus*, while *Micrococcus* was only represented for Gram-positive group on brass. Unlike the other materials, no Gram-negative strains were identified on copper. *Micrococcus* and *Staphylococcus* belonging to Gram-positive group were identified from copper coupons.

Table 3 shows the generic composition of MHB isolated from various materials. Both Gram-positive and Gram-negative groups were isolated from PVC, SS and brass. However, only Gram-positive groups, especially of the endospore-forming genus *Bacillus* sp. and a non-spore forming genus *Propionibacterium* were recorded on copper coupons. In general, the bacterial strains isolated from all the materials showed that Gram-positive groups were dominant. Only genus *Bacillus* was commonly encountered on all the materials studied. Under Gram-positive group, apart from the genus *Bacillus*, other genera identified were *Micrococcus*, *Staphylococcus* (PVC), *Synecoccus* (SS), *Micrococcus* (brass) and *Propionibacterium* (copper), while the genus *Pseudomonas* belonging to Gram-negative group was recorded from PVC and SS. However, this could not be recovered from brass and copper. The Gram-negative genus *Vibrio* was recorded from brass coupons.

Table 4 shows the levels of Mn in sea water and biofilm. Among the four types of coupons studied, PVC exhibited highest Mn value of 8775 ng/g, while lowest Mn value of 206 ng/g was recorded from brass coupons. As low as 0.7 μg/l of Mn was observed in sea water.

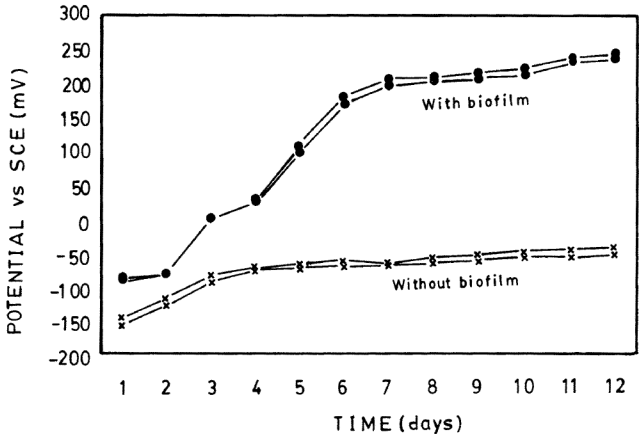

Figure 1. Potential measurements for SS316 in the presence and absence of biofilm.

Table 1. Average counts of total heterotrophic bacteria and manganese-oxidizing heterotrophic bacteria in immersed coupons PVC, copper, stainless steel and brass

Material immersed	Duration (in months)	Total heterotrophic bacteria CFU/cm^2	Manganese-oxidizing heterotrophic bacteria CFU/cm^2
PVC	1	3.07×10^8	2.36×10^8
SS	1	3.40×10^5	1.07×10^5
Brass	6	3.90×10^6	1.84×10^6
Copper	6	2.04×10^2	1.02×10^2

Table 2. Generic composition of heterotrophic bacterial isolates and their per cent occurrence (in parenthesis) in various materials

	PVC	SS	Brass	Copper
No. of isolates	25	20	20	10
Gram-positive	15 (60)	15 (75)	5 (25)	10 (100)
Gram-negative	10 (40)	5 (25)	15 (75)	–
Staphylococcus	10 (40)	5 (25)	–	5 (50)
Micrococcus	5 (20)	10 (50)	5 (25)	–
Pseudomonas	5 (20)	–	20 (50)	–
Acinetobacter	5 (20)	5 (25)	5 (25)	–
Proteus	–	–	5 (25)	–

Table 3. Generic composition of manganese-oxidizing heterotrophic bacterial isolates and their present occurrence (in parenthesis) in various materials

	PVC	SS	Brass	Copper
No. of isolates	18	15	12	15
Gram-positive	12 (66.66)	12 (80)	6 (50)	15 (100)
Gram-negative	6 (33.33)	3 (20)	6 (50)	–
Bacillus	3 (16.66)	9 (60)	3 (25)	12 (80)
Micrococcus	6 (33.33)	–	3 (25)	–
Staphylococcus	3 (16.66)	–	–	–
Synecoccus	–	3 (20)	–	–
Pseudomonas	6 (33.33)	3 (20)	–	–
Propioni bacterium	–	–	–	3 (20)
Vibrio	–	–	6 (50)	–

Eashwar *et al.*[24] reported the absence of ennoblement on SS exposed to irradiated sea water. They suggested that some photo-labile catalyst could be the cause for the loss of ennoblement. It is well known that MnO_2 is photo-labile. Under constant light conditions, photo-chemical reaction takes place and leads to reduction of MnO_2. Therefore under such conditions, ennoblement (increase of potential) is not possible. In the present study, SS316 ennobled as high as 242 mV. This could be due to the deposition of MnO_2 on SS by manganese-oxidizing bacteria.

Micro-organisms are important agents in the oxidation and deposition of iron and manganese[25]. The manganese-oxidizing group is a phylogenetically diverse assemblage which is characterized by the ability to catalyse the oxidation of divalent soluble Mn (II) to insoluble manganese. In the present study also, cocci and rods were represented in MHB, which have not been reported earlier from biofilms. Manganese-oxidizing bacteria are ubiquitous; they can be isolated from nearly any habitat. It is known that habitats containing high levels of Mn and those in which Mn-cycling is an active process tend to have a high number of Mn-oxidizing bacteria.

In the present study, occurrence of both Gram-positive and Gram-negative bacteria was noted from manganese-oxidizing bacterial isolates. However, spore-forming genus *Bacillus* was commonly seen on all the substrates studied, where the Mn concentration in biofilms was also abundant (Table 4). This observation further confirms the earlier report by Eisenstadt *et al.*[26], who showed that

Table 4. Heavy metal concentration in sea water (µg/l) and biofilm (ng/g) on various materials

Coupon	Duration (days)	Manganese concentration
Sea water	–	0.77
PVC	30	8775
SS	30	1112
Brass	180	206
Copper	180	1179

Bacilli require more Mn for sporulation than during vegetative growth. Besides *Bacillus* sp., it has been reported that *Oceanospirillum*, *Vibrio*[27,28] and *Pseudomonas*[29] are involved in manganese-oxidation. In the present study the involvement of *Pseudomonas* and *Vibrio* belonging to the Gram-negative group, in Mn oxidation could be seen on selected substrates. This finding explains the specificity of the substrate. According to Devrind *et al.*[30] the cytochrome-c maturation operon (CCM) is involved in oxidation of manganese by *Pseudomonas putida*. Further, on copper coupons, only Gram-positive groups, especially of the endospore-forming genus *Bacillus* sp. and a non-spore forming genus *Propionibacterium* were identified. The absence of other groups could be explained due to the toxic nature of the substrate and two genera identified could be regarded as copper-tolerant groups. The level of manganese is low in brass compared to copper coupons. It can be explained that the predominating positive genera (*Bacilli* sp.) is forced to develop adaptation against copper toxicity by forming spores which may accumulate more manganese. Even though higher bacterial population was recorded on brass coupons, the accumulation of manganese by predominantly Gram-negative strains is poor compared to positive strains.

Bacillus sp., which is identified as a copper-resistant species in the present study is known to involve in the corrosion of chromium–nickel, steel and carbon steel in sea water or fresh water-based cooling systems, where normally chlorine is used as a biocide. The effectiveness of chlorine as a biocide would be in question, especially for the tolerant species. Therefore, the present study would form a basis for the manufacturers of both antifouling anode and biocides, to come out with suitable products to control metal accumulating/resistant species like *Bacillus*.

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Genotoxicity study in lymphocytes of workers in wooden furniture industry

D. Elavarasi*, V. Ramakrishnan, T. Subramoniam*, A. Ramesh†, K. M. Cherian** and C. Emmanuel**,#**

*Department of Biotechnology, University of Madras, Guindy Campus, Chennai 600 025, India
†Department of Genetics, University of Madras, Taramani Campus, Chennai 600 113, India
**Institute of Cardio-Vascular Diseases, Madras Medical Mission, 4 A Mugappair, Chennai 600 050, India

We have evaluated the extent of genetic damage (frequencies of sister chromatid exchange (SCE) and micronuclei) in blood lymphocytes and found a correlation between exposure period and genetic damage, in workers in the wooden furniture industry.

Venous blood samples were drawn from thirty non-smoking workers exposed to wood dust and thirty non-smoking controls. Lymphocytes were cultured for 72 h and at the end of the culture time the cells were harvested, stained and coded for blind scoring. Damage to the genetic material was evaluated by counting the number of SCEs in 100 metaphases and the frequency of micronuclei in 1000 binucleated cells. Mitotic and proliferative rate indices were also determined.

Environmental studies showed a dust level of 0.30 mg/m^3 (particle size 2.5 microns). The mean micronuclei frequency was significantly higher in the exposed group (17.2%; SD = 1.17, $P < 0.001$; range 13–21.1) compared to the controls (7.54%; SD = 1.6, $P < 0.001$; range 5–10.5). The mean value of SCEs was also higher (7.17, SD = 1.80) in the exposed workers than in the controls (6.71, SD = 1.56), but the difference was not statistically significant. A positive correlation existed between the duration of exposure and micronucleus frequency (correlation coefficient, $\gamma = 0.64$) and SCE (correlation coefficient $\gamma = 0.47$). These results indicate that occupational exposure to wood dust causes genetic damage, which may lead to health hazards like cancer, with or without other risk factors.

OCCUPATIONAL exposure to various dust particles is a common cause of respiratory disorders like wheezing and acute and chronic bronchitis in wood-processing factory employees, grain workers and feed-mill employees[1]. Several independent groups have suggested health risk associated with chronic occupational exposure to wood dust[2–7]. These studies reported the occurrence of lower and upper respiratory diseases such as alveolitis allergica, bronchial asthma, aspergillomycosis and rhinitis. The genotoxicity of wood dust can be evaluated using the sister chromatid exchange (SCE) and micronuclei test (MNT). However the frequency of micronuclei serves as the best quantitative measure for structural and numerical chromosome

#For correspondence. (e-mail: mmmbits@vsnl.com)